Biochar provides a safe and value-added solution for hyperaccumulating plant disposal: A case study of *Phytolacca acinosa* Roxb. (*Phytolaccaceae*)

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Abstract

In this work, an innovative approach using biochar technology for hyperaccumulator disposal was developed and evaluated. The heavy metal enriched *P. acinosa* biomass (PBM) was pyrolyzed to produce biochar (PBC). Both PBM and PBC were characterized with X-ray diffraction (XRD) for crystal phases, scanning electron microscopy (SEM) for surface topography, and analyzed for elemental composition and mobility. The results revealed that whewellite, a dominant crystal form in biomass, was decomposed to calcite after pyrolysis. Elemental analysis indicated that 91-99% total non-volatile elements in the biomass were retained in the biochar. The toxicity characteristic leaching procedure (TCLP) results revealed that 94.6% and 0.15% of total Mn was extracted for biomass and biochar, respectively. This suggests that mobility and bioavailability of Mn in biochar was much lower relative to pristine biomass. Batch sorption experiment showed that excellent removal of aqueous silver, lead, cadmium, and copper ions can be achieved with PBC. Findings from this work indicated that biochar technology can provide a value-added solution for hyperaccumulator disposal.

Keywords: Phytomanagement; Black Carbon; Charcoal; Waste Recycling; Environmental Remediation

1. Introduction

Heavy metal in soils may originate from weathering of soil minerals, air deposition, solid waste application, irrigation and mining (Evanko and Dzombak, 1997). Heavy metals are persistent in soils and may threaten soil quality, plant growth, and human health (Chalot et al., 2012; Chen et al., 2016). The traditional *in-situ* and *ex-situ* remediation techniques to remove heavy metals from the contaminated soil are well documented, such as immobilization, extraction, which are usually low-efficient and not cost-effective (Evanko and Dzombak, 1997; Ahmad et al., 2014; Mosa et al., 2016). Hyperaccumulators could tolerate high concentrations of toxic metals and play important ecological functions in the environment (Boyd, 2004; Mok et al., 2013). Phytoremediation as an *in-situ* and inexpensive approaches gains popularity in past few decades as a promising technique to concentrate and remove heavy metals from mild and moderately contaminated soils (Kramer, 2010; Mok et al., 2013). However, the disposal of residual biomass has been a problematic issue which has not been well resolved (Sas-Nowosielska et al., 2004).

Previous work demonstrated several potential approaches for biomass disposal, including direct disposal, chemical extraction of biomass, composting and combustion (Chalot et al., 2012). With respect to these traditional treatment approaches, additional problems may arise. For example, on one hand, the direct disposal and composting could cause secondary pollutions by releasing the immobilized contaminants; on other hand, combustion or burning of biomass can release black carbon aerosols containing heavy metals in the atmosphere, which harm human health, reduce precipitation and increase release of greenhouse gases (Chalot et al., 2012).

As an alternative, pyrolysis under oxygen limited conditions was proposed to reduce the bulk volume of biomass and concentrate the heavy metals in very stable carbon enriched

biochars (Lievens et al., 2008). With well controlled and usually low pyrolysis temperatures, the common heavy metals can be well concentrated and immobilized in the biochar matrix (Lievens et al., 2008). For examples, compared to the traditional combustion at high temperatures where Pb and Zn were found in considerable amount in gas fractions, both of them were not detected in oil, gas and tar fractions during oxygen-free pyrolysis (Chalot et al., 2012). Thus, pyrolysis may minimize the loss and maximize sequestrations of the heavy metals, especially those with relatively low melting points (Koppolu et al., 2003; 2004).

Biochars can be used as soil amendment and heavy metal sorbents (Ahmad et al., 2014; Inyang et al., 2016; Rajapaksha et al., 2016). Although pyrolysis techniques have been reported to concentrate and fix heavy metal in biochars, not much work have been done to investigate the leaching potential and ecotoxicity of these heavy metals sequestered in biochars. In order to avoid further damage due to heavy metal exposure, the bioavailability of heavy metals in biochars needs to be evaluated. Seed germination tests are usually adopted to assess heavy metal phytotoxicity to plants (Di Salvatore et al., 2008; Yao et al., 2013; Bae et al., 2014). Previous work has already successfully used seed germination assay tests to evaluate the phytotoxicity of biochars to seed germination and early growth.

In addition, there is no detailed discussion with respect to the phase changes of heavy metals in biomass when converting to biochars. Previous work confirmed that heavy metals may mainly complex with oxalate, but the main minerals in biochars was not ascertained. Because of different solubility, the properties of heavy metal-bearing minerals can affect their bioavailability and leaching ability. Thus, to address these concerns, we used *P. acinosa* (*Phytolacca Americana*) biomass as a model plant species to investigate the mobility of heavy metals and its phoytoxicity to seed germination.

P. acinosa was reported as a hyperaccumulator for several heavy metals, especially for manganese (Mn) (Dou et al., 2009). Xue et al (2003), for the first time, identified a perennial herb Phytolaccaceae as a new Mn hyperaccumulator plant. They (Xue et al., 2003; Xue et al., 2004) collected the plants from the Mn-tailing and observed Mn content in leaf tissue ranged from 1.2-1.9 percent on dry mass basis, which exceeded the criterion for the Mn-hyperaccumulator plant (Kramer, 2010). Excellent hyper-accumulating capacity and large biomass yields of P. acinosa facilitate high removal efficiency for heavy metals. However, the Mn concentrations in other tissues such as stem, fruit and roots are low, which bring down the overall Mn concentration in whole plants. Since it is not practical to separate Mn-concentrated leaf tissue from other parts due to operation costs, we evaluated the possibility of pyrolyzing the whole plants to simulate the situations in real-world practices.

In this work, P. acinosa derived biochars were made and possible phase changes from biomass to biochars were determined. The objectives of this work were: (1) to prepare and characterize P. acinosa biochar, (2) to investigate mobility of heavy metals using The toxicity characteristic leaching procedure (TCLP), (3) to determine the removal capacity of the biochar for heavy metals in aqueous solutions, and (4) to study the effects of both BM and BC on seed germination.

2. Materials and Methods

2.1 Reagents

Lead nitrate ($\text{Pb}(\text{NO}_3)_2$), silver nitrate (AgNO_3), copper chloride (CuCl_2), cadmium nitrate ($\text{Cd}(\text{NO}_3)_2$) of analytical grade were purchased from Fisher Scientific and Sigma-Aldrich

(United States). Ultrapure water (Nanopure water, Barnstead) denoted as DI water (18.2 MΩ) was used in the experiments.

2.2 Biochar preparation

P. acinosa (*Phytolacca Americana*) biomass was collected from the same sites as described in a previous study (Xue et al., 2004). The whole plants including leaf, stem and root tissues were homogenized and freeze-dried, and crushed for later use. To prepare biochars, the oven-dried biomass was ground into powder and pyrolyzed at peak temperature of 600 °C for one hour (at heating rate of 10 °C per min) in a N₂ gas purged tube furnace (MTI, Richmond, CA) (Wang et al., 2015b). The pristine biochars (PBC) was stored for later use.

2.3 Characterization of PBM and PBC

Both PBM and PBC were prepared with AOAC method (AOAC, 1990) and the elemental composition (metals and phosphorus) was determined with an inductively coupled plasma-atomic emission spectrometer (ICP-AES, Perkin-Elmer Plasma 3200). Total carbon (C) and nitrogen (N) content in the biochar samples was analyzed with a CHN elemental analyzer (Carlo-Erba NA-1500). Specific surface area and pore volume was determined using density functional theory (DFT) with a NOVA 1200 analyzer. Thermal stability (TG) and derivative thermogravimetric (DT) analysis of PBM and PBC was analyzed with Mettler Toledo's TGA/DSC1 analyzer, with combustion temperature between 25 and 700 °C under air or nitrogen atmosphere. Surface morphology of both PBM and PBC was examined with a scanning electron microscope (SEM) using a JEOL JSM-6400 Scanning Microscope. The binding energy of C1s and O1s were determined with an X-ray photoelectron spectroscopy (XPS) with a PHI 5100 series ESCA spectrometer (Perkin-Elmer). Crystals was obtained with a CuKα radiation X-ray diffractometer (XRD) (Philips Electronic Instruments).

2.4 TCLP extraction

The mobility of inorganic elements in PBM and PBC was analyzed using toxicity characteristic leaching procedure (TCLP) following the USEPA Method 1311 (USEPA, 1990; Yao et al., 2012). The experiment followed the procedures of USEPA Method 1311 (USEPA, 1992). Briefly, 0.5 g PBM or PBC was extracted by 10 ml extraction solution (solid/solution=1:20, pH=4.9) in standard extraction vessels prepared following Yao et al (2012). The solutions were filtered and elemental analysis was performed for filtrate with ICP-AES.

2.5 Heavy metal removal and seed germination experiments

Heavy metal removal: The PBC was investigated for its sorption capacity for cationic heavy metals such as silver (Ag), lead (Pb), cadmium (Cd) and copper (Cu) at concentrations of 400, 330, 100 and 100 mg L⁻¹, respectively, with the sorbate concentration of 2g L⁻¹. The vessels were shaken at 40 rpm on a rotary shaker at room temperature (22 ± 0.5 °C). The suspensions were immediately filtered through 0.22 µm pore size nylon membrane filters (GE cellulose nylon membrane). All treatments were triplicated and the means of the three replications were reported with standard deviation.

Seed germination experiment (Yao et al., 2013): Both biomass and biochars were evenly spreaded on the filter paper and wetted with deionized (DI) water. Twenty uniform grass seeds (*Brown Top Millot*) was sowed on wet filter paper loaded with or without biomass or biochars in a 100 ml covered plastic container. The treatments include PBM, PBC, and washed PBC (PBCw) at 0.1 g in each container, with DI as control (CK). To obtain PBCw, 0.5g PBC was soaked in 50 ml DI water and shaken at 40 rpm on a rotary shaker for 2 h before filtering through 0.45 nm nylon filter paper. The filtered PBC was oven dried at 80 °C overnight. Each treatment has three replicates.

3. Results and Discussion

3.1 Characterization

About 63 % weight loss was recorded when PBM was pyrolyzed at 600 °C under oxygen free conditions. The C and N contents in PBC were 1.55 and 1.22 times that in PBM, respectively. The concentrations of non-volatile elements in PBM were increased by 2.41-2.75 times in PBC, which suggested that 89-97% of these elements were retained in the PBC (Table 1). This increment was due to the loss of the volatile components when the non-volatile components remained during the pyrolysis. The lower Mn concentrations in the homogenized biomass than in leaf tissues (Xue et al., 2004) was associated with dilution effects. The Mn concentrated leaf tissue was diluted by large mass fraction of stem and roots with much lower Mn concentration. The weight loss of PBM was evidenced by the TG/DTG curves of the biomass in N₂ atmosphere, which occurred at 217 and 297 °C (Figure 1).

The minerals in PBM were transformed after pyrolysis. The XRD results revealed that the peaks of PBM and PBC diffractograms corresponded to whewellite ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$) and calcite (CaCO_3), respectively (Figure S1, supporting information). Similarly, Dou et al. (2009) reported that oxalate was a major organic form in *P. acinosa*, which may precipitate with the metals. The complexation of oxalate with Ca was further consolidated by unidentifiable TCLP extractable Ca in PBM (Table 2). During the pyrolysis process, whewellite was decomposed in which H₂O and CO were gradually lost, and eventually transformed into calcite (Chang and Huang, 1997) (Figure S1, supporting information).

Thermal stability of both PBM and PBC was evaluated by combustion in air up to temperature of 700 °C (Figure 1). The PBM underwent several weight loss phases when

combusted in the air atmosphere, where rapid loss occurred at 217, 275 and 390 °C representing 0.42, 0.63, and 0.78 % °C⁻¹. The slight weight loss at 168 °C might be attributed to loss of water in whewellite (Chang and Huang, 1997). At 217 and 275 °C, the weight loss might be associated with the changes of chemical phase and physical states of lignocellulosic materials in biomass, while weight loss at 390 °C was induced by combustion of organic components (Sebio-Punal et al., 2012). Different inflammability was found in PBC, where the rapid weight loss occurred at 372 °C at rate of 2.23 % °C⁻¹. This is mainly related to the loss of the combustible components in biochars, since the calcite would not decompose below 600 °C (Rodriguez-Navarro et al., 2009).

Surface topography as revealed by SEM imaging (Figure S2, supporting information) indicated that PBC had more porous surface structure than PBM. The phase and crystallinity transformation of lignocellulosic biomass could occur during pyrolysis (Keiluweit et al., 2010). This may contribute to increased surface area and pore volume in PBCw, which were about 40 and 25 times higher than those in PBM (Table 1). The lower porosity of PBM was due to the occupancy of impurity in char pores.

The XPS C1s and O1s spectra suggested the change of both C and O-containing functional groups (Figure S3, supporting information). The C1s at binding energies of 284.50-284.60 eV, 285.44-285.88 eV and 288.17 eV corresponded to graphitic or aromatic, hydroxyl and carboxyl functional groups, respectively (Dong et al., 2014). The O1s at binding energies of 532.78-532.93 eV can be assigned to hydroxyl and carboxyl functional groups and 531.46 eV to O in carbonate. This confirmed the change of functional groups after pyrolysis (Dong et al., 2014).

3.2 Environmental hazard and application

The TCLP results showed that all non-volatile elements except Mg in PBM were more mobile than those in PBC (Table 2). Concentrations of TCLP-extractable Mn, Zn and Pb in PBM were several hundred times greater than those in PBC. Besides, less than 0.15% of total Fe, Mn, Zn and Pb were extracted in PBC, compared to 17.9, 94.6, 52.4 and 14.2% for PBM. These results implied that PBC increased the stability of heavy metals relative to PBM, thus reduced bioavailability of these toxic metals. However, Ca was not leached in PBM and only 8.77% was extracted in PBC, which suggests Ca existed in different mineral forms with varying solubility in PBM and PBC (Figure S1, supporting information).

To investigate ecotoxicological effects of PBC, seed germination experiment was performed. The shoot height in both PBM and PBC treatments was significantly inhibited compared to CK (Figure 2). There was no significant difference between control and PBCw. The inhibition effects of both PBM and PBC on seed germination caused concerns of using biochars (Figure 2). However, the excellent growth in PBCw treatment suggested that the inhibition effects of PBC may be mainly associated with the osmotic damage from leached salts, including considerable amounts of Mg, P, Na and K from unwashed PBC (data not shown) (Panuccio et al., 2014). Because there is less leaching of heavy metals in the PBC, it is safe to use PBC in soil when excess salts can be quickly diluted and leached away in soil solutions. Besides, biochars are excellent soil amendment which can improve soil health and fertility (Bell and Worrall, 2011; Lehmann et al., 2015; Novak et al., 2016).

Biochars can also be used to remediate heavy metal contamination (Mohan et al., 2007). The increased surface area and pore volume provide more niche for contaminants (Table 1). The sorption study showed that the PBC has very good removal capacity for common heavy metals

(Figure 3). The removal capacities for contaminants were as follows: Ag (198.3 g kg^{-1}) > Pb (152.0 g kg^{-1}) > Cu (47.7 g kg^{-1}) > Cd (43.7 g kg^{-1}). The sorption capacity of these pristine biochars for these heavy metals is better than most pristine chars and similar as those treated chars, i.e., Cu $35.8\text{--}53.8 \text{ g kg}^{-1}$ (Zuo et al., 2016), $1.47\text{--}6.81 \text{ g kg}^{-1}$ (Jiang et al., 2016), $7.44\text{--}50.2 \text{ g kg}^{-1}$ (Park et al., 2016) and references therein), $29.4\text{--}33.3$ (Bogusz et al., 2015); Cd, $41.7\text{--}72.4$ (Goswami et al., 2016), 3.85 (Yap et al.), $32.6\text{--}35.7$ (Bogusz et al., 2015); Pb, $3.9\text{--}162.75$ (Yap et al.) and references therein), $0.88\text{--}53.6$ (Ding et al., 2016) and references therein.

Because the dominant minerals on the resulting PBC is calcite, the extraordinary heavy metal removal may be attributed to sorption and precipitation (Aziz et al., 2008). Thus, the PBC has minimal environmental hazard, and showed great potential for application in environmental remediation.

3.3 A practical solution to disposal of heavy metal hyper-accumulated plants

The hyperaccumulators can colonize on highly contaminated soils, which benefit soil in such ways as reducing contamination and improving soil properties. In an effort to find an appropriate approach for disposal of hyperaccumulator, the pokeweed as a Mn hyperaccumulator was used as a model plant species and pyrolyzed and converted to biochars. Although such beneficial effects of common biochars on environment as carbon sequestration, soil amendment and heavy metal removal have been well discussed elsewhere (Zimmerman et al., 2011; Cernansky, 2015), the potential use of heavy metal-concentrated biochars are still questionable. The dilemma mainly lies in immobilization efficiency of heavy metals, and environmental safety of direct disposal of as-prepared biochars. The results in this study are very optimistic in these aspects (Table 1 and 2), which clearly demonstrated that biochars can efficiently immobilize

heavy metals, and displayed no ecotoxicity and environmental safety. Moreover, biochars offer a value-added solution for hyperaccumulator disposal due to their high sorptive ability for heavy metals.

Once combusted in air, the C in biomass is converted to carbon dioxide (CO₂), which contributes to global warming (Frolicher et al., 2014). Heavy metals can be vaporized at high temperatures (Koppolu et al., 2004). A previous attempt indicated that the combustion of phytoremediation wood in a boiler resulted in low recovery of Zn (less than 60%) in bottom ash and 48% emitted as fly ash or biomass burning aerosol (Chalot et al., 2012). Thus, the direct combustion may not be appropriate to concentrate the heavy metals in ash. In an attempt to sequester heavy metals in pyrolysis product (biochars), the results looked not promising. It was found that only 67% Zn and 83% Cd were immobilized in the biochars fraction when pyrolyzed at 673 K (Lievens et al., 2008). In contrast, our results showed over 90% recovery of heavy metals in biochars was accomplished. This may be related to the pyrolytic conditions, such as temperature regimes, protecting gases, and the elemental composition of biomass. The detailed discussion on effects of biochar properties and crystal composition on elemental mobility should be further investigated.

The sequestered heavy metals in biomass could be released into soils. Staaf and Berg (1982) showed that 75% Mn in biomass was released into soils after one year incubation. Our results also suggested that about 95% Mn in PBM can be extracted with TCLP procedure. That is, if the biomass returns to soil, the heavy metals in the hyperaccumulator would be much more mobile and bioavailable compared to PBC (Table 2). The rapid release of heavy metals from biomass when incorporated into soil are mostly related to both rapid decomposition of organic substances in biomass and lower stability of metal-containing compounds. On one hand, a large

fractions of C from plant residues would be decomposed to yield a large amounts of humic substances (Struecker et al., 2017), which can complex and mobilize the heavy metals in the biomass (Chen et al., 2002; Refaey et al., 2017). Instead, biochars are recalcitrant from degradation (Cernansky, 2015) and characterized with lower labile C fractions (Zimmerman et al., 2011) and rich functional groups for metal retention (Dong et al., 2014). For example, biochars are able to immobilize nickel, chromium, and Mn in heavy metal contaminated serpentine soil (Bandara et al., 2016). On other hand, the metal compounds in the biomass can be converted to their more stable forms. For example, whewellite ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$) in the biomass was converted to relatively more stable calcite (CaCO_3) in this study. The FeCl_3 and MnCl_2 were transformed to Fe oxide and various forms of Mn oxides during the charring process, respectively (Zhang et al., 2013; Wang et al., 2015a). Thus, the biochars can be able to protect the heavy metals from leaching. However, to date, there is no report to discuss the leaching potential of heavy metals from hyperaccumulator and its derived biochars. TCLP uses a buffered solution to stimulate leaching, and widely used to evaluate the leaching potential from solid materials. Therefore, TCLP is implemented to determine the potential hazard of both biomass and biochars. The TCLP extraction for biochars revealed that almost no Mn, Zn and Pb were leached out of biochar matrix. Thus, direct disposal of PBM can be hazardous due to enormous leaching, while the leaching potential is greatly cut down when converted into biochars.

Overall, the direct disposal of heavy metal enriched plant species, i.e., hyperaccumulator, may cause rapid release of heavy metals to environment. However, hyperaccumulator-derived biochars are able to prevent rapid C loss and immobilize heavy metals. In addition, they can be used as high-efficient sorbents for heavy metals.

4. Conclusions

This study showed that non-volatile metals were condensed in *P. acinosa* biochars during pyrolysis. The XRD and SEM analysis revealed that major minerals (whewellite) in PBM were decomposed into calcite in PBC. TCLP analysis revealed that heavy metals in PBC were much less mobile compared to pristine PBM. The PBC exhibited extraordinary removal capacity for selected metals (Ag, Pb, Cu, and Cd) in aqueous solutions. Thus, slow pyrolysis for biochar purpose is suitable for disposal of metal hyperaccumulating plants by reducing the volume of biomass, condensing the heavy metals, and reducing the leaching potential of heavy metals.

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450 **Tables**

451 Table 1. Elemental composition (% , mass), surface area and pore volume of *P. acinosa* biomass (PBM) and *P. acinosa* derived
452 biochar (PBC)

	C	N	Ca	Mg	Al	P	Fe	Mn	Zn	DFT Surface area m ² g ⁻¹	DFT pore vol (des. leg) cc g ⁻¹
	%, mass based										
PBM	37.37	2.83	1.32	0.56	0.05	0.65	0.04	0.04	0.07	11.8	0.004
PBC	57.78	3.45	3.45	1.35	0.14	1.60	0.11	0.10	0.18	10.0	0.004
PBCw	-	-	-	-	-	-	-	-	-	468.8	0.099

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455 Table 2. The toxicity characteristic leaching procedure (TCLP) extractable elemental contents in *P. acinosa* biomass (PBM) and *P.*
456 *acinosa* derived biochar (PBC)

	TCLP elemental extraction (µg/g)											
	Ca	Mg	Al	P	Fe	Mn	Cu	Cr	Ni	Cd	Pb	Zn
PBM	ND	5920.38	153.98	7338.16	75.74	381.63	17.29	0.74	4.46	0.60	4.55	371.24
PBC	3025.68	6647.12	6.72	585.22	0.70	1.52	2.10	0.40	1.17	0.17	0.00	0.78
	Percent elements extracted (%)											
PBM	ND	100.00	28.73	100.00	17.90	94.58	100.00	17.80	74.25	48.51	14.18	52.40
PBC	8.77	49.39	0.49	3.65	0.06	0.15	7.41	2.26	5.36	46.11	0.00	0.04

457 Note: ND=not detected

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459

460 **Figure captions**

461 **Figure 1.** Thermogravimetric (TG) (a) and derivative thermogravimetric (DT) (b) analysis for *P.*
462 *acinosa* (PBM) and biochars (PBC)

463 **Figure 2.** Effects of *P. acinosa* (PBM) and biochar (PBC) on seed germination. Treatments
464 include CK: DI water; PBM: 0.1 g biomass in each petri dish; PBC: 0.1 g biochar in
465 each petri dish; PBCw: 0.1 g washed biochar. Each bar represents standard deviation
466 of treatment means.

467 **Figure 3.** The sorption capacity of heavy metals (Ag, Pb, Cu, Cd) by *P. acinosa* derived biochars
468 (PBC). Bars represent standard deviation of treatment means.

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470

471 **Figure 1**

476 **Figure 2**

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480 **Figure 3**

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